IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	MANSOUR SAMADPOUR
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Dear Dr. Lu,

As a way of introduction, I am a microbiologist/molecular epidemiologist working in academia as an assistant professor. My field of specialty is the use of molecular techniques for subtyping microbial pathogens. What led to the discovery of the Micro Restriction Fingerprinting (MRF) method was a tremendous need to develop a rapid, sensitive, and low cost methodology for DNA fingerprinting of microbial pathogens. To fit the description we needed a method that would not require expensive electrophoresis equipment (such as the ones used in the PFGE method, for about $10,000-$20,000 per unit, or the Riboprinter for $200,000), the method had to be fast, accurate, reproducible, and sensitive without requiring hybridization. It took me more than a year to develop the MRF method. Examination of the prior art by me and others had provided no clues. The quality of the patterns generated by the chromosomal DNA restriction methods, including the Preston et al's report were so low that no one was even pursuing their work to optimize it. The discovery of the MRF method was quite accidental, while I was trying to find better enzymes to conduct the Lambda phage RFLP analysis of E. coli O157, the RFLP patterns of the six base cutter enzymes were easily seen on agarose gels, but I could not see the RFLP patterns generated by the use of a few of the four base cutters. In the next set of experiments I doubled the amount of DNA used for the four base cutters (used 2 micrograms), this again did not result in seeing a banding pattern, at 4 micrograms with one of the enzymes I could barely see bands, raising the levels to 10-20 micrograms suddenly produced a very differentiative banding pattern with *Sau*3a. The pattern was so clear and distinctive that there was no need to conduct

hybridization. It took several more months to develop and test the method and document that it is as useful or even superior to all subtyping methods available to date, mainly by the virtue of the fact that it is easy to perform, low cost and the data generated has high epidemiological relevance. As some one who is relatively skilled in the arts and works with the most skilled people in the art internationally, nothing about this method was obvious nor was it available. Even at this point none of the experts in the field who know of the existence of the method and the types of restriction enzymes that we use in MRF, have been able to reproduce it without obtaining detailed instructions and protocols.